J Nanostruct 14(4): 1077-1081, Autumn 2024

RESEARCH PAPER

Chitosan/ HAP Nanoparticles: Antibacterial Study

Essa D. Alhtheal [1], Ali K. Shakir [2], Ammar M. Hamza [3], Hussam Sami Awayid [4]*

[1] *Center of nanotechnology& advanced materials, University of Technology, Iraq*

[2] *College of science for women, University of Babylon, Babylon, Iraq*

[3] *Civil Engineering Department, University of Technology, Iraq*

[4] *Middle Technical University, Kut-Technical Institute, Iraq*

ARTICLE INFO

Article History:
Received 17 July 2024
Accepted 28 September 2024
Published 01 October 2024

Keywords:
Antibacterial
Biomedical
HAP
Nanomaterials

ABSTRACT

Combination of organic polymer with Hydroxyapatite (HAP) credible composites would be used widely in biomaterial and environmental engineering. The surface chemical characterization of chitosan/ Hydroxyapatite nanoparticles (HAPNPs) was investigated by X-ray diffraction (XRD) and Fourier transform infrared (FTIR). Morphology images of composite samples were detected by Transmission electron microscopic (TEM) and Field emission scanning microscope (FESM). These results were confirmed homogeneous structure, and interaction between the chitosan and HAP. The antibacterial responses to the hydroxyapatite/chitosan matrix coatings were studied as a function of hydroxyapatite concentration. The results of antibacterial test showed that the antimicrobial activity was proportional to the concentration of hydroxyapatite nanoparticles. The synthesis process for the proposed nano hydroxyapatite is also very simple, low cost, and friendly environmental. Moreover, All the above advantages indicated the intriguing potential of the samples can be used in biomedical and practical wastewater treatment applications.

How to cite this article
Alhtheal E., Shakir A., Hamza A., Awayid H. Chitosan/ HAP Nanoparticles: Antibacterial Study. J Nanostruct, 2024; 14(4):1077-1081. DOI: 10.22052/JNS.2024.04.009

INTRODUCTION

The World Health Organization reports were Diagnosed that the major health problem is dental caries, especially among disadvantaged social groups worldwide. This disease affects both sexes, all races and age groups, and different social and economic classes. Dental caries causes pain and sadness, and it imposes a relatively high financial burden on families. There are about 700 different bacterial species in human oral microbiota. Antimicrobials such as ampicillin, chlorhexidine, quaternary ammonium compounds and metronidazole are used to prevent tooth decay, but they have several side effects such as tooth staining, diarrhea, increased calculus formation and changes in bowel flora. Therefore, new methods for prevention and probably better management of this important challenge are needed. In recent years, the use of natural nontoxic alternatives for the control and prevention of dental caries has emerged. Chitosan is a natural polymer with specific properties, including nontoxicity, antimicrobial activity and biodegradability, which has attracted great attention for some years. Prior studies have described the antimicrobial potential of chitin, chitosan and their derivatives [1-3].

However, application of micro and nano crystalline chitosan structure have a clear

* *Corresponding Author Email: tab_mar2002@yahoo.co.uk*

impedance to the dissolution in neutral pH as well biodegradation prolongation due to high crystallinity of the Chitosan bio-composites. Hydroxyapatite (HAP), ($Ca_{10}(PO_4)3OH$), is present in mammalian as major inorganic compound and highly recognized and used for its bio-compatibility. The calcium phosphates are perfect for bone replacement, promote rates of resorption/replacement so appropriate which have been proven by the Environmental Physiology and the osseoconductive. The Hydroxyapatite is very similar to natural bone than other calcium phosphate formula (like ß-TCP, ($Ca_3(PO_4)_2$)) [4-5]. The performance of a polymer medical device depends on bulk and surface material properties [6].

Many researchers are work on development of nano HAP composite with biopolymer, because of their identification to bone matrix when coupled with biological and mechanical performances [7]. Biopolymers materials are very widely applications in the fields of biomedical and orthopedic, because of their biocompatibility and biodegradability properties [8]. Many composited containing HAP/polymer such as HAP/ collagen [9], HAP/chitosan [10], HAP/ collagen/poly(lacticacid) [11], HAP/ alginate/collagen [12], HAP/gelatin [13] were used for bone tissue engineering. Chitosan and curcumin with HAPNPs showed reproductive toxicity via its ameliorative effects on the fertility, testicular tissue functions, antioxidant system and hormonal status [14].

In this study low cost technique was used to produce HAPNPs and mixing with chitosan at different percentage. The morphology testing shows unique structure for CS/HA NPs and cluster for HAPNPs alone. It showed a good antibacterial activity against E-coli and and Staphylococcus aureus bacterial.

MATERIALS AND METHODS

Raw Materials and Characterization

The raw materials used in this work are: Chitosan (grade of deacethylation 0.85, MW 110 kDa, Calcium nitrate ($Ca(NO_3)_2.4H_2O$) tetrahydrate, diammonium hydrogen phosphate ($(NH_4)2HPO_4$ were purchasing from various suppliers. All solvents and reagents were used without any additional purification. The morphology and diameter of the HAPNPs and/or CS/ HAPNPs were examined by using TE FEI technical G2 Split Biotic transmission electron microscope at (120 kv) and Field Emission Scanning Electron Microscopy, model Quanta 200FEG, which is configured to operate at 120 Kv with various magnification level. The crystallinity and crystal phase for HAPNPs and/or CS/ HAPNPs were studied by,using [Rigaku X-ray-diffracto-meter, 6000, Shimadzu, Japan). The physical properties and chemical structure were characterized by using FTIR [FTIR 8400S,Shimadzu, Japan] and UV- Vis spectroscopy [AA 6300, Shimadzu, Japan]. The antimicrobial tests were made in vitro.

Method

First, the procedure used for Hydroxyapatite (HA) nanoparticles preparation in this study have followed according to our previous study [15]. In briefly, Hydroxyapatite nanoparticles (HAPNPs)

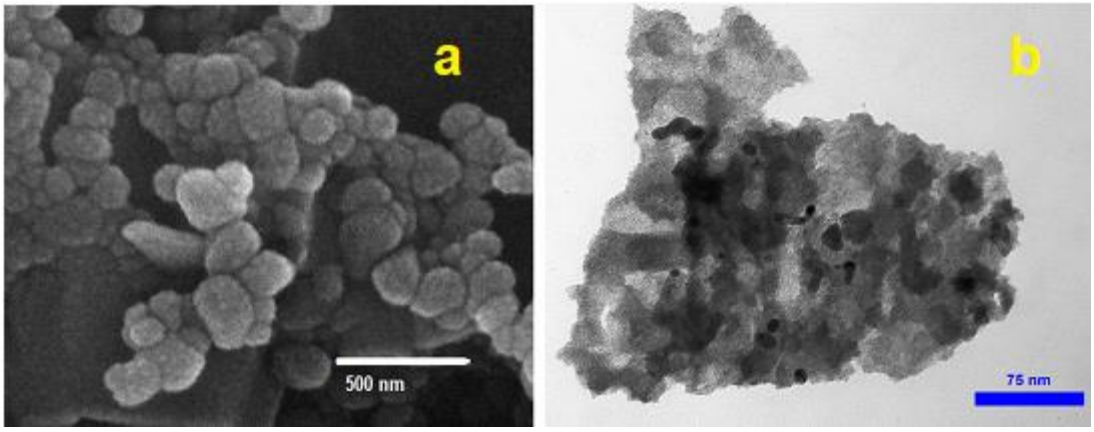

Fig. 1. (a) FESEM of HAPNPs & (b) TEM of CS/HAPNPs (15wt%)

were obtained from diammonium hydrogen phosphate and calcium nitrate tetrahydrate precursors, Ammonia used in control of the acidic function at 11. The use of ultrasound to study the impact on the particle sizes. Chitosan were mixed with HA nanoparticles in Distilled water with different percentage [5%, 10%, and 15% respectively]. The morphology were examined by mean of TEM, FESEM, XRD, and FTIR. CS/HA nanoparticles solutions were fabricated by using the agar diffusion test in same way as describe from Ammar Hamza et el. [16]. E. coli and Staphylococcus aureus, which both of them are positive gram are used to study The antibacterial properties of CS/HA nanoparticles composite.

RESULT AND DISCUSSION

The nanoparticles which get from HAP by using ultrasonic technique and Fig. 1a shows the morphology of HAPNPs. The results of obtained hydroxyapatite powders showed almost spherical particles with narrow size distribution. Average size of HAP particles is approximately ~ 90 nm as shown in Fig.1a. The FESEM images indicated Individual spheres with agglomerated and porosity. We think the formation like a sphere for HAPNPs are due to the high level of correlation between ultrasonic waves and HAP molecules [17]. Fig. 1b show the transmission electron microscope (TEM) for CS/HAPNPs 15wt%. It was showed a unique shape of the prepared material,

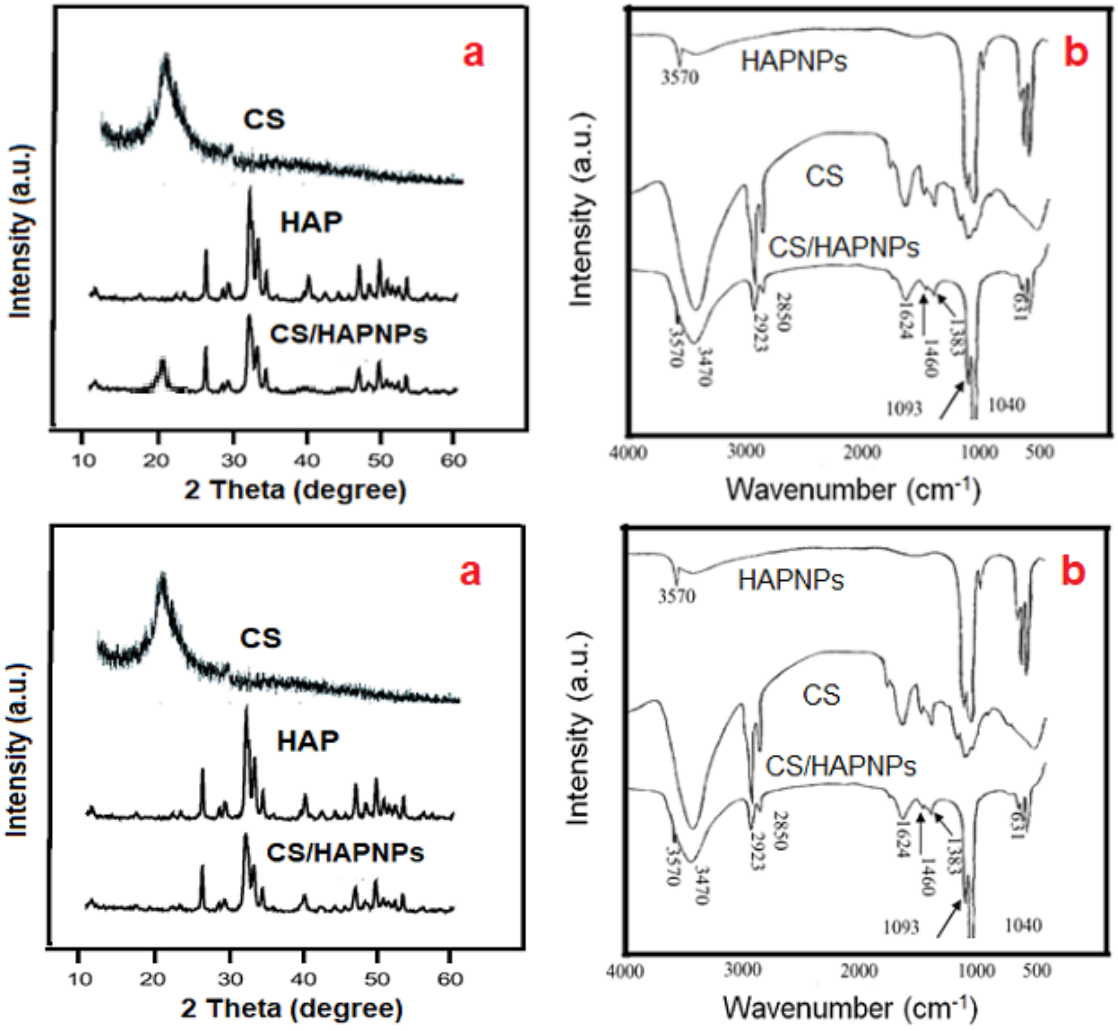

Fig. 2. (a) XRD & (b) FTIR of CS, HAPNPS, and CS/HAPNPs

which was in the form of nanoparticles for HAP composed with chitosan. It is clear that the original HAP are highly complicated particles with average diameters of ~35 nm. We think that two reasons caused the diameters of HAPNPs are less, as it composited with CS; first, the cluster was formed for HAP molecules. Second, CS-HAPNPs composite prepared by gel-formation indicated that the HAPNPs are relatively dispersed over a wide area in the chitosan matrix. The TEM results of HAPNPs were suggested that can be homogeneously incorporated with Chitosan.

The XRD patterns of the CS, HAPNPS, and CS/HAPNPs 15wt% samples are summarized in Fig. 2a. The result of XRD were confirmed by the Power Diffraction File (HAP: Card No. 090432; CS: Card No. 391894). It indicated that, the broad diffraction characteristic peak that noticed around 20° was correspond to CS (20.305°, 21.290°), when Chitosan alone or mixing with HAPNPs. The sharp peaks that noticed at around 31.8° and 25.9° assigned to the HAPNPs (31.773°, 25.879°). XRD patterns indicated the presence of peaks for both chitosan and HAPNPS with approximately no change in the position of angle (2θ). FTIR spectra are indicated that absorption bands at 3470, 2923, 1624, and 1383 cm^{-1} for chitosan molecular, and at 3470 and 3570 cm^{-1} is corresponded to the stretching and vibration of the lattice OH^- ions in chitosan molecular and Hydroxyapatite nanoparticles [18]. The bands at 631 cm^{-1} are for the v1 mode of characteristic bands $PO4^{-3}$, and absorption bands at 1093/1040 cm^{-1} for the v3 symmetric P-O stretching vibration were observed. The absorption bands for $HPO4^{-2}$ were appeared at 1393 $cm^{-1}$and 1460 cm^{-1} as shown in Fig. 2b. These results pointed out the presence of HAPNPS in the Chitosan samples, in accord with the XRD results [19-20].

Fig. 3 (a and b) showed the weight percentage of HAPNPs have activity against E. coli and Staphylococcus aureus because the antimicrobial activity is concerned with compounds that make locally killed microbial or decreased slowly their growth. Nowadays, most of current antimicrobial agents are produced from chemically modified natural compounds [21-22]. A ruler was used to measure Zone of inhibition, which is defined as the average diameter of the area around the discs that stops the growth of bacteria. It was observed

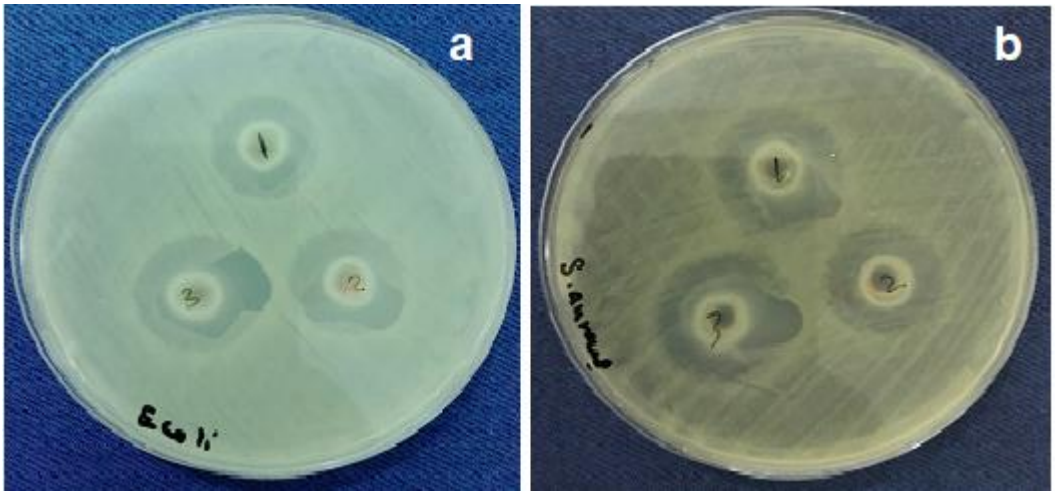

Fig.3. (a)E-Coli & (b) Staph, aureus with different percentage of CS/HA (5, 10, 15 wt%)

Table1. Inhibited zone of CS/HAPNPs for different concentration

No	CS/HAPNPs %	E-Coli	Staph. aureus
1	5	18	21
2	10	21	24
3	15	24	26.5

that the CS/HAPNPs synthesized have antibacterial activities against Staphylococcus aureus more than E. coli as shown in Table 1. As the weight percentage of HAPNPs increased, the inhibition zones are increased for both bacterial.

CONCLUSION

The synthesis of HAP with spherical and a roughly ~35 nm particle size in aqueous solution has been achieved in the presence of the chitosan which are adopted by TEM and FESEM equipment. Also, they showed hierarchical ordering compositionally from inorganic (HAP) to organic (chitosan), which suggested very interesting and novel biomedical applications such as drug delivery devices, hybrid tissue engineering and scaffolds. The XRD and FTIR spectrum conformed the structure for composites. Antibacterial test was highly dependent on the concentration of a nano-hydroxyapatite as shown in the inhibited zone. These results show a strong biomaterials property for CS/HAP nanoparticles.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Aliasghari A, Khorasgani MR, Khoroushi M. The Effect of Vinegar, Rose Water and Ethanolic Extract Green Tea Against Oral Streptococci, an In Vitro Study. Journal of Probiotics and Health. 2017;05(04).
2. Petersen PE, Lennon MA. Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. Community Dentistry and Oral Epidemiology. 2004;32(5):319-321.
3. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: A state of the art review. Int J Food Microbiol. 2010;144(1):51-63.
4. Pighinelli L, Kucharska M. Properties and Structure of Microcrystalline Chitosan and Hydroxyapatite Composites. J Biomater Nanobiotechnol. 2014;05(02):128-138.
5. Pighinelli L, Kucharska M, Wiśniewska-Wrona M, Gruchała B, Brzoza-Malczewska K. Biodegradation study of microcrystalline chitosan and microcrystalline chitosan/β-TCP complex composites. Int J Mol Sci. 2012;13(6):7617-7628.
6. Wawro D, Pighinelli L. Chitosan fibers modified with HAp/β-TCP nanoparticles. Int J Mol Sci. 2011;12(11):7286-7300.
7. Victor Abere D, Modupe Oyatogun G, Lawan Rominiyi A, Ayodeji Abioye A, Emmanuel Akinwole I, Magnus T I, et al. Investigation of Physicochemical and Mechanical Property of UTAN Granites for Building Applications. American Journal of Mining and Metallurgy. 2017;4(1):37-44.
8. El-Meliegy E, Abu-Elsaad NI, El-Kady AM, Ibrahim MA. Improvement of physico-chemical properties of dextran-chitosan composite scaffolds by addition of nano-hydroxyapatite. Sci Rep. 2018;8(1):12180-12180.
9. Kikuchi M, Itoh S, Matsumoto HN, Koyama Y, Takakuda K, Shinomiya K, et al. Fibrillogenesis of Hydroxyapatite/ Collagen Self-Organized Composites. Key Eng Mater. 2003;240-242:567-570.
10. Yamaguchi I, Tokuchi K, Fukuzaki H, Koyama Y, Takakuda K, Monma H, et al. Preparation and microstructure analysis of chitosan/hydroxyapatite nanocomposites. J Biomed Mater Res. 2001;55(1):20-27.
11. Table of Contents. Journal of Bioactive and Compatible Polymers. 2004;19(1):1-1.
12. Han X, Chen S, Cai Z, Zhu Y, Yi W, Guan M, et al. A Diagnostic and Therapeutic Hydrogel to Promote Vascularization via Blood Sugar Reduction for Wound Healing (Adv. Funct. Mater. 14/2023). Adv Funct Mater. 2023;33(14).
13. . Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2005;72B(2).
14. Abd El-Aziz El-Gendy M, Helmy El-Dabbah F, Ibrahim Hassan A, Abd El-Naby Awad N. Hepatotoxic and cardiotoxic effects of testosterone enanthate abuse on adult male albino rats. Al-Azhar Medical Journal. 2021;50(2):1335-1348.
15. K. Al-Khateeb I, M. Hamza A, J. Abduljabar E. Nano-hydroxyapatite formation by using ultrasonic. Journal of University of Anbar for Pure Science. 2017;11(3):56-61.
16. Hamza AM, Alhtheal ED, Shakir AK. Enhancement the Efficiency of ZnOnanofiber mats antibacterial Using Novel PVA/Ag nanoparticles. Energy Procedia. 2017;119:615-621.
17. Prekajski M, Jokic B, Kalijadis A, Maletaskic J, Stankovic N, Lukovic J, et al. Synthesis of silver doped hydroxyapatite nanospheres using Ouzo effect. Processing and Application of Ceramics. 2016;10(3):169-174.
18. Anee TK, Palanichamy M, Ashok M, Sundaram NM, Kalkura SN. Influence of iron and temperature on the crystallization of calcium phosphates at the physiological pH. Mater Lett. 2004;58(3-4):478-482.
19. Hu X, Ji J. Synthesis and self-assembly of sulfonated hyperbranched polyethers and their biocompatibility. Acta Polymerica Sinica. 2009;009(8):828-833.
20. Koumoulidis GC, Katsoulidis AP, Ladavos AK, Pomonis PJ, Trapalis CC, Sdoukos AT, et al. Preparation of hydroxyapatite via microemulsion route. Journal of Colloid and Interface Science. 2003;259(2):254-260.
21. Wang X, Albahrani Y, Pan M, Levitt J. Skin simulators for dermatological procedures. Dermatol Online J. 2015;21(11).
22. Ali ZC, Shakir S, Aslam TM. Perceptions and use of technology in older people with ophthalmic conditions. F1000Research. 2019;8:86-86.